EXPRESSION AND REGULATION OF THE STRESS HORMONE PRECURSOR PROTEIN, PROOPIOMELANOCORTIN (POMC) IN MERINO SKIN

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The identification of molecules responsible for the stress-induced physiological shutdown of wool follicles is of central importance in developing methods for the prevention of wool breaks and tender fleeces. One such candidate is the 29-33kDa protein POMC, which in the pituitary is cleaved enzymatically to yield the opioid peptide, bendorphin, the adrenocortical trophic hormone (ACTH), and the pigmentation regulatory peptide aMSH. This protein has been found in many peripheral tissues including mouse skin, where expression is highest during the active growth phase of the hair cycle (Slominski *et al.* 1996). Our previous studies have localised POMC immunoreactivity to the outer root sheath of wool follicles, sebaceous glands and the epidermis of Merino skin (Behrendt *et al.* 1993). In this report we have characterized the POMC protein and identified a POMC gene product in skin using the polymerase chain reaction (PCR) which is regulated by altering circulating concentrations of stress hormones.

Merino ewes (n=7) were actively immunised against ACTH1-24ovalbumin in Freunds complete adjuvant intramuscularly in weeks 1, 6, 8, 35 and 37 of the study. ACTH-specific antibody titres measured by ELISA reached 29,798±5,858 at week 35 and were boosted to 225,170±48,741 by week 38. Skin biopsies (1cm) were collected by trephine and total RNA extracted. cDNA was generated by reverse transcription from equal quantities of total RNA from ACTH-immune and control sheep skin and appropriate specific oligonucleotide primers designed from the POMC gene to amplify by PCR a 550bp product corresponding to the POMC coding sequence. The presence of the POMC protein was detected with a rabbit polyclonal anti-ACTH primary antibody by Western blotting analysis using 75µg of total protein from both immune and control skin.

A single 550bpPCR product was detected in ACTH-immune and control skin. This band was attenuated in ACTH-immune skin sampled when ACTH-antibody titres were low in week 35, but conversely much higher than in control skin when ACTH antibody titres were boosted in week 38. Parallel changes in POMC expression were observed in pituitary tissue collected from single pituitaries collected from animals at the same time points. The Western blot analysis detected a single 29kDa translated POMC protein in both ACTH-immune and control skin.

We have demonstrated the transcription of a specific mRNA for POMC and its translation into an intact POMC protein, which is not processed into its constituent peptides in the skin. The integrity of this protein suggests that pro-hormone convertases required for the generation of the biologically active peptides are absent from skin. However the parallel regulation of pituitary and skin POMC mRNA levels in response to ACTH immunisation suggests that circulating stress hormone levels may play an important role in the integration of the stress response in central and peripheral tissues.

The positive identification of this gene and its product in the skin provides further evidence for an intact neuroendocrine system for the modulation of stress responses being present in skin, which is regulatable by central control mechanisms. This regulation may be directed through the traditional endocrine signal pathway.

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